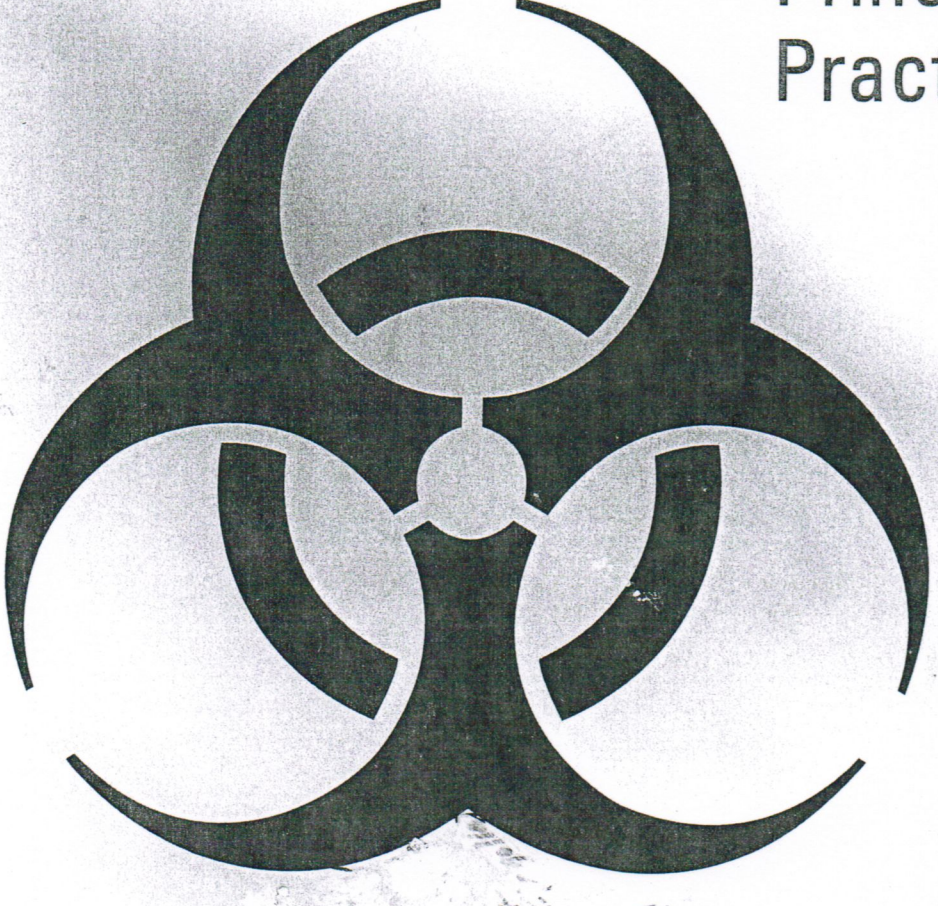
4TH EDITION

Biological Safety

Principles and Practices



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Laboratory, Growth Chamber, and Greenhouse Microbial Safety: Plant Pathogens and Plant-Associated Microorganisms of Significance to Human Health

ANNE K. VIDAVER, SUE A. TOLIN, AND PATRICIA LAMBRECHT

3

Several years ago, interkingdom pathogenic specificity was rarely recognized (Starr and Chatterjee, 1972). We now know that an increasing number of the organisms, and occasionally even the same strains of an organism, can infect both plants and humans. Commonalities in gene sequences and function among pathogens of plants, animals, and humans are no longer surprising (Cao et al., 2001; Alfano and Collmer, 2004; Govan and Vandamme, 1998; Govan, et al., 1996; Gorbalenya, et al., 1989; Rodriguez-Villalobos et al., 2002; Tan, 2002). In addition, some plant-associated microorganisms which may prevent plant disease nevertheless can cause human allergies or disease. Such biocontrol agents are regulated commercially by the U.S. Environmental Protection Agency for environmental risks but not for risks to humans. Hence, it is prudent to assess safety issues with respect to human exposure to plant-associated microorganisms, including viruses, for laboratory and confined uses. Keeping with the purpose of this book, our focus is on risk characterization and mitigation of worker exposure during culturing, inoculation of plants, and diagnosis of plant pathogens known to affect human health.

For bacteria, commonalities among plant, animal, or human pathogens are most evident in type III secretion pathways (Alfano and Collmer, 2004; Hueck, 1998) and virulence factors in pseudomonads (Govan and Vandamme, 1998; Rahme et al., 1995; Tan, 2002). In most cases, virulence or pathogenicity factors in common still await discovery. In fungi, commonalities are at the structural, morphological, biochemical, and genetic levels (Hall et al., 1999; Procop and Roberts, 1998). In viruses, gene sequences may be highly homologous and similarities in genomic functionality are known (Gorbalenya et al., 1989; Hohn and Fütterer, 1997; Toh et al., 1983).

The emergence or reemergence of human diseases caused by microorganisms is due to many factors (Vidaver, 1996). In humans, for example, both *Burkholderia cepacia* and *Pseudomonas aeruginosa* have become important pathogens in cystic fibrosis; both are also an infrequent cause of infection in non-cystic fibrosis patients (Holmes et al., 1999; Vikram et al., 1999). The emergence of newly identified fungal pathogens and the reemergence of previously uncommon fungal diseases of humans (mycoses) are attributed to an increase in the number of susceptible individuals, such as bone marrow and organ transplant recipients, cancer patients being treated with chemotherapy, critically ill persons, very-low-birth-weight infants, and persons with certain other infections, notably human immunodeficiency virus (Dixon et al., 1996). Clinically relevant mycoses may occur in healthy, immunocompetent individuals as well (Pontón et al., 2000; see also chapter 8 on mycotic diseases).

The many texts and manuals that deal with methods of working with plant pathogens do not provide any cautions or statements with respect to potential risk to human health (Blanchard and Tater, 1981; Burgess and Liddell, 1983; Dhingra and Sinclair, 1995; Fahy and Persley, 1983; Hampton et al., 1990; Hickey, 1986; Kahn and Mathur, 1999; Klement et al., 1990; Lelliot and Stead, 1997; Razin and Tully, 1983; Rechcigl and Rechcigl, 1997; Saettler et al., 1989; Schaad, 2001; Schneck, 1982; Singleton et al., 1992; Tuite, 1988; CDC/NIH, 1999; NIH, 2002; U.S. Department of Agriculture [USDA], 1992), except for a one-page statement in a recent handbook (Ritchie, 2002). Nor in medical texts is there any caution about the potential for occupational exposure to plant pathogens or plant-associated organisms, other than as allergens (Horner et al., 1995).

There are increasing numbers of plant-pathogenic microbial organisms associated with human diseases or maladies (Table 1). Of the more than 300 reported species of fungi isolated from humans with infectious systemic diseases (Taylor et al., 2001), at least 40 are known plant pathogens. Of these, all but two are in the phylum Ascomycota, popularly known as ascomycetes. Multiple species in the genera *Alternaria*, *Aspergillus*, *Bipolaris*, *Colletotrichum*, *Curvularia*, and *Fusarium* have been implicated in human disease. Ten additional genera are represented by one species each. This suggests specificity in evolution of pathogenicity as well as susceptibility in humans, which may simply be the ability of humans to meet the nutritional and asexual reproductive capacity or other characteristics of these fungi. Many plant-pathogenic fungi are associated with mycotoxicoses, but these are not considered in Table 1 because entry is via consumption of contaminated foods and unlikely to occur in a laboratory setting (Bennett and Klich, 2003).

Of the more than 500 species of bacteria isolated from human infections (Taylor et al., 2001), 12 are also known as plant pathogens or as biocontrol agents associated with plants. Reports of human infections for 22 bacterial species are listed in Table 1. Of these species, only six are gram positive, with three being *Bacillus* spp. The rest are gram negative and include multiple species in the genera *Agrobacterium*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Serratia*, and single species of *Pantoea*, *Stenotrophomonas*, and *Xanthomonas*. Homologues of bacterial genes coding for virulence or pathogenicity factors in common between crossover pathogens are becoming better known (Table 2) (Alfano and Collmer, 2001; Tan, 2002). For access to the literature, as well as to journals covering plant diseases or associations, the reader is referred to common texts in plant pathology (Agrios, 2005; Lucas and Dickinson, 1998). Only a few direct references combine plant and human microbial associations.

No report to date implicates plant viruses in human disease. Anecdotally, plant virologists have been known to produce antibodies to viruses with which they have worked, and have shown allergic sensitivities following the inhalation of viral aerosols. However, it is unlikely that a plant virus could infect and become viremic in humans. Specific proteins on the outer surface of animal and human viruses bind to cellular receptors, proteins on the outer surface of cells, inducing penetration, translocation of viral components to intracellular sites, and replication (Mettenleiter, 2002). In contrast, plant viruses have no known cellular receptor-binding proteins and require a mechanically induced or insect-induced wound in a host cell to facilitate penetration. Theoretically, a needle stick from a hollow-bore needle attached to a syringe containing a plant pathogen could also provide a mode of entry into the human host.

Given the small size of viral genomes, and similarity in functions, it is not surprising to see many closely related sequences between plant and human viruses (Table 2). There has been one suggestion that a plant nanovirus had recombined with picorna-like viral RNA to form circoviruses infecting vertebrates (Gibbs and Weiller, 1999). The host switch recombinational event is postulated to have occurred in a vertebrate when it was exposed to sap from a nanovirus-infected plant. Thus, plant viruses may pose a risk in the event that there is an open wound or percutaneous injury through which a virus may enter. The end result, however, is likely to be the creation of a new virus which may or may not cause disease in the infected host. Dimitrov (2004) and Baranowski et al. (2001) reviewed virus entry and the evolution of cell recognition by viruses and suggested that minimal changes in viral genomes may trigger changes in receptor usage for virus entry; neither review considered plant viruses.

RISK ASSESSMENT AND BLs

As the NIH guidelines note (NIH, 2002), risk assessment is ultimately a subjective process. By the standard that agents are not associated with disease in healthy adult humans, almost all the plant pathogens and plant-associated microorganisms are in NIH risk group 1, for which biosafety level 1 (BL1) is usually recommended. However, as noted in Table 1, a few plant pathogens should be viewed as more problematic in that some strains of some species may infect both immunocompromised hosts and, more rarely,

TABLE 1 *Continued*

Taxon[a]	Plant disease/association	Human disease
Stenotrophomonas maltophilia	Plant-associated and plant pathogen (Suckstorff and Berg, 2003)	Bacteremia and respiratory tract infections (Denton and Kerr, 1998; Goss et al., 2004)
Xanthomonas campestris pv. campestris	Black rot of crucifers and wilt and blight stump rot of broccoli, cabbage, cauliflower, brussel sprouts, kale, mustard, radish, rutabaga, sunflower, stock, and turnip (Agrios, 2005; Westcott, 2001)	Bacteremia (Li et al., 1990)
Fungi[b]		
Phylum *Ascomycota*		
Alternaria alternata	Wide host range. Causes leaf spots, blights, damping off, and stem and fruit rots (Westcott, 2001). Black mold of tomato, leaf spot and ear and root rot of maize, blight of foliage and pod of pea, seedling foliage blight of sugarcane (Rott and Comstock, 2000), black point on wheat, dark flecks on *Dendrobium*, fruit spot on papaya, and leaf spot on garden bean	Phaeohyphomycosis (Duffill and Coley, 1993), mycotic keratitis, cutaneous (Ono et al., 2004) and visceral infections, and osteomyelitis
Alternaria tenuissima	Strawberry fruit rot (Howard and Albregts, 1973), leaf spot of broad bean (Honda et al., 2001), leaf spot of high bush blueberry (Milholland, 1995), leaf spot of *Amaranthus hybridus* (Blodgett and Swart, 2002), and leaf spot of papaya	Phaeohyphomycosis (Romano et al., 1996; Romano et al., 1997), sinusitis, and ulcerated cutaneous and visceral infections (Rossman et al., 1996)
Aspergillus candidus	Decay of apple fruits (Thind et al., 1976), root rots	Cerebral aspergillosis (Linares et al., 1971), cutaneous aspergillosis, endocarditis, endophthalmitis, hepatosplenic aspergillosis, meningitis, myocarditis, onychomycosis, osteomyelitis, otomycosis, pulmonary aspergillosis (Krysinska-Traczyk and Dutkiewicz, 2000), and sinusitis
Aspergillus flavus	Pathogen and saprophyte; has many hosts and causes such diseases as ear and kernel rot of maize (St. Leger et al., 2000; Smart et al., 1990), yellow mold of peanut (Pitt et al., 1991), and boll rot on cotton (Brown et al., 1992) Biopesticide: *A. flavus* strain AF36 (Arizona Cotton Research and Protection Council, Phoenix, Ariz.), a non-toxin-producing strain registered (EPA[c]) on cotton fields in Texas and Arizona for control of strains of *A. flavus* which produce aflatoxin; *A. flavus* strain NRRL 21882, registered for use in peanut crops to control aflatoxin-producing strains of *A. flavus* (Circle One Global, Inc., Shellman, Ga.)	Systemic aspergillosis (Yamada et al., 1998) and endocarditis (Rao and Saha, 2000)

(TABLE 1 *continued*)

TABLE 1 Taxa of pathogens and saprophytes of plants associated with human disease or maladies

Taxon[a]	Plant disease/association	Human disease
Bacteria		
Gram positive		
Bacillus megaterium	White blotch of wheat and bacterial wetwood of poplar and elm (Murdoch and Campana, 1983)	Oral mucosal inflammation (Rubinstein and Pedersen, 2002)
Bacillus circulans	Date palm disease (Leary and Chun, 1989)	Peritonitis (Berry et al., 2004), serious nongastrointestinal infections in animals, and diarrheal enterotoxin production in human cells (Rowan et al., 2003; Deva and Narayan, 1989)
Bacillus pumilus	Bacterial blotch of immature Balady peach (Saleh et al., 1997); registered biocontrol agent, strain GB34 Yield Shield; (Gustafson, Plano, Tex.) for control of soilborne fungal pathogens causing root disease in soybean	Oral mucosal inflammation (Rubinstein and Pedersen, 2002; Suominen et al., 1999)
Clostridium butyricum	Wetwood of poplar (Schink et al., 1981) and disease of hornbeam (Gvozdiak et al., 1976)	Necrotizing enterocolitis in babies (Howard et al., 1977)
Clostridium histolyticum	Plant associated	Gas gangrene (myonecrosis) and necrotic lesions (Brazier et al., 2004)
Rathayibacter toxicus (syn. *Clavibacter toxicus*)	Gummosis of cereals (Riley and Ophel, 1992)	Death of livestock associated with consumption of *Rathayibacter*-infected annual ryegrass (Riley and Ophel, 1992); human disease speculative (Edgar, 2004)
Gram negative		
Agrobacterium radiobacter (syn. *Rhizobium radiobacter*)	Plant-associated bacteria, rhizosphere; registered biocontrol agent for crown gall, strain K84 (Galltrol A; AgBioChem Inc., Orinda, Calif.) in fruit, nut, and ornamental nursery stock and strain K1026 (Nogall; Bio-Care Technology Pty Ltd., Somersby, New South Wales, Australia) for control of crown gall on fruit and nut trees, caneberries, roses, and other ornamentals	Opportunist pathogen (Edmond, 1993). Bacterial endophthalmitis (Miller et al., 1996); bacteremia (Southern, 1996), endocarditis (Plotkin, 1980), peritonitis (Melgosa Hijosa et al., 1997), and urinary tract infections (Namdari et al., 2003; Dunne et al., 1993)
Agrobacterium tumefaciens (syn. *Rhizobium tumefaciens*)	Agent of crown gall with wide host range (Moore and Warren, 1979; Westcott, 2001)	Peritonitis (Ramirez et al., 1992; Alnor et al., 1994), bacteremias (Southern, 1996), and urinary tract infection (Hulse et al., 1993)
Burkholderia cepacia	Sour skin of onion (Burkholder, 1950; Yohalem and Lorbeer, 1997) and cavity disease of *Agaricus bitorquis* (Gill and Cole, 1992; Alameda and Migrucci, 1998); phytoremediation (Glick, 2004) and endophyte (Hinton and Bacon, 1995)	Bacteremia (Woods et al., 2004); pulmonary complex (De Boeck et al., 2004; Courtney et al., 2004); serious respiratory pathogen in cystic fibrosis patients (Wigley and Burton, 1999; Govan et al., 1996); bacteremia, cardiac cirrhosis and cellulitis (Lau et al., 1999); and endophthalmitis (Pathengay et al., 2004)

(TABLE 1 *continued*)

TABLE 1 *Continued*

Taxon[a]	Plant disease/association	Human disease
Burkholderia gladioli	Slippery skin of onion (Kishun and Swarup, 1981); decay of *Gladiolus* spp., *Iris* spp., and rice; leaf spot and blight of *Asplenium nidus* (Chase et al., 1984); and bacterial disease of *Dendrobium* sp. orchid (Chuenchitt et al., 1983)	Bacteremia (Shin et al., 1997), pneumonia (Ross et al., 1995), and cervical adenitis (Graves et al., 1997)
Enterobacter cloacae	Wetwood on elm, internal decay of onion (Bishop and Davis, 1990), and rhizome rot of edible ginger (Nishijima et al. 2004); biocontrol agent (Punja, 1997; Wilson et al., 1987; Watanabe et al., 2000)	Septicemia and respiratory tract infections (Jochimsen et al., 1998) and gas gangrene (Fata et al., 1996)
Erwinia persicina (syn. *Erwinia nulandii*)	Necrosis in fruits, vegetables (Hao et al., 1990), necrosis of bean pods and seeds (Brenner et al., 1994)	Urinary tract infection (O'Hara et al., 1998)
Klebsiella pneumoniae	Endophyte; many plant hosts, including maize (Dong et al., 2003)	Pneumonia (Prince et al., 1997), bacteremia (Kang et al., 2004), and meningitis (Tang and Chen, 1994)
Klebsiella variicola	Plant-associated on banana, rice, sugarcane, and maize (Rosenblueth et al., 2004)	Bacteremia and urinary tract infection (Rosenblueth et al., 2004)
Pantoea agglomerans (syn. *Enterobacter agglomerans, Erwinia herbicola*)	Pathogen of *Wisteria* and onion; wetwood of elm; black flesh of pineapple and grapefruit; spot disease and frost damage on corn, soy, and clover; and disease of millet (Frederickson et al., 1997). Saprophyte	Nosocomial/opportunistic infections (Bennett et al., 1995) and septic arthritis (Kratz et al., 2003)
Pseudomonas aeruginosa	Onion rot (Cother et al., 1976) and *Arabidopsis* rot	Burn wound infections and pneumonia (Rahme et al., 1995; Johansen et al., 1998; Vikram et al., 1999) and meningitis, bacteremia, and sepsis (Torii et al., 2003)
Pseudomonas fluorescens	Registered biocontrol for *Erwinia amylovora* (Johnson and Stockwell, 1998) on apple, cherry, almond, peach, and pear (Blight Ban A506; Frost Technology Corporation, Burr Ridge Ill.); frost protection on fruit crops, almond, tomato, and potato to reduce frost-forming bacteria on leaves and blossoms (Frostban; Frost Technology Corporation)	Bacteremia (Hsueh et al., 1998)
Pseudomonas putida	Plant saprophyte with potential for application in biological control of plant pathogens, bioremediation, and production of bioplastics (Nelson et al., 2002)	Nosocomial infections (Lombardi et al., 2002); meningitis (Ghosh et al., 2000); and bacteremia, pneumonia, and sepsis (Torii et al., 2003)
Serratia ficaria	Plant associated (biological cycle) (Grimont et al., 1979)	Organ infections (Anahory et al., 1998) and endophthalmitis, gall bladder empyema, and septicemia (Badenoch et al., 2002)
Serratia marcescens	Alfalfa crown and root rot (Lukezic et al., 1982), cucurbit yellow vine disease (Bruton et al., 2003), and endophytic colonization of rice (Gyaneshwar et al., 2001)	Respiratory tract infections, urinary tract infections, and bacteremia (Ostrowsky et al., 2002) and conjunctivitis, endocarditis, meningitis, and wound infections (Su et al., 2003)

(TABLE 1 *continued*)

TABLE 1 *Continued*

Taxon[a]	Plant disease/association	Human disease
Aspergillus glaucus	Corn ear and kernel rot (Nyvall, 1979)	Cerebral, cutaneous, hepatosplenic, and pulmonary aspergillosis; endocarditis; endophthalmitis; meningitis; myocarditis; onychomycosis; osteomyelitis; otomycosis; and sinusitis (O'Shaughnessy et al., 2004)
Aspergillus niger	Black mold of peanut (Nyvall, 1979) and onion (Tanaka and Nonaka, 1977) and maize ear rot (Nyvall, 1979)	Aspergilloma (fruiting body in tissue), otomycoses (Mishra et al., 2004), and pulmonary aspergillosis (Yamaguchi et al., 1992)
Aspergillus oryzae	Saprophyte and mycotoxin producer (Geiser et al., 2000)	Necrotizing scleritis (Stenson et al., 1982) and bronchopulmonary aspergillosis (Akiyama et al., 1987)
Aureobasidium pullulans	Russet of apple fruit (Matteson Heidenreich et al., 1997) and d'Anjou pear (Spotts and Cervantes, 2002) and stem break and browning of flax	Various opportunistic mycoses, pulmonary mycoses, scleritis (Gupta et al., 2001), and phaeohyphomycosis (Kaczmarski et al., 1986)
Bipolaris australiensis	Leaf spot and crown and root rot of turfgrass, (http://www.apsnet.org/online/common/comment/turfgrass.asp	Phaeohyphomycosis, allergic and chronic sinusitis, keratitis, endophthalmitis (Chalet et al., 1986), endocarditis, osteomyelitis, meningitis, encephalitis, peritonitis, and pulmonary infection (Flanagan and Bryceson, 1997)
Bipolaris hawaiiensis	Leaf and culm lesions on Callides Rhodesgrass (Sonoda, 1991) and Bermuda grass disease (Pratt, 2001)	Endophthalmitis, phaeohyphomycotic orbitopathy, sinuitis, and granulomatous encephalitis (Morton et al., 1986)
Bipolaris spicifera	Leaf spot of cotton (http://www.apsnet.org/online/common/names/cotton.asp)	Phaeohyphomycosis (McGinnis, et al., 1992), fungal endarteritis (Ogden et al., 1992), meningitis (Latham, 2000), and peritonitis (Bava et al., 2003)
Chaetomium globosum	Disease in tomato (Geraldi et al., 1980) and infection of barley roots	Cerebral phaeohyphomycosis (Anandi et al. 1989) and onychomycosis (Hattori et al., 2000)
Cladosporium oxysporum	Leaf spots and blights of many plants and leaf spot of pepper (Hammouda, 1992)	Phaeohyphomycosis (Romano et al., 1999) and pneumonia (Yeghen et al., 1996)
Colletotrichum coccodes	Black dot of tomato (Dillard and Cobb, 1997) and potato (Andrivon et al., 1998)	Phaeohyphomycosis (O'Quinn et al., 2001)
Colletotrichum gloeosporioides	Anthracnose on many fruits and plantation crops (Westcott, 2001), including anthracnose of papaya leaves (Dickman and Alvarez, 1983) and avocado, poplar, aspen, and cottonwood shoot blight, fruit rot on apple and berries of coffee, and dieback of citrus	Keratitis (Yamamato et al., 2001) and phaeohyphomycosis (O'Quinn et al., 2001)
Coniothyrium fuckelii	Stem blight, dieback, and canker of *Rosa* spp. and strawberry *(Fragaria)* and black root rot and cane blight of *Rubus* spp. (Heimann and Boone, 1983)	Mycotic keratitis (Laverde et al., 1973) and liver infection (Kiehn et al., 1987)
Curvularia brachyspora	Leaf spot disease of *Rosa* spp. (Kore and Bhide, 1976)	Necrotizing cutaneous infection (Torda and Jones, 1997) and mycotic keratitis (Marcus et al., 1992)
Curvularia clavata	Leaf spot of maize (Mandokhot and Chaudhary, 1972)	Invasive sinusitis and cerebritis (Ebright et al., 1999) and human skin infection (Gugnani et al., 1990)

(TABLE 1 *continued*)

TABLE 1 *Continued*

Taxon[a]	Plant disease/association	Human disease
Curvularia geniculata	Banana leaf spot (Meredith, 1963) and melting out of turfgrasses (Westcott, 1990)	Mycotic keratitis (Georg, 1964) and maduromycotic mycetomas in animals (Bridges, 1957)
Curvularia lunata	Leaf rot of rice (Lakshmanan, 1992), leaf spot of bentgrass (Muchovej and Couch, 1987), melting out of turfgrasses (Westcott, 2001), leaf spot of maize (Ito et al., 1979), and leaf spot of cotton (Gour and Dube, 1975)	Cerebral phaeohyphomycosis (Carter and Boudreaux, 2004), systemic cutaneous infection (Tessari et al., 2003), and allergic fungal rhinosinusitis (Taj-Aldeen et al., 2004)
Curvularia pallescens	Leaf spot of sugarcane (Rao et al., 1992), leaf spot and ear rot of maize (Lal and Tripathi, 1977), brown spot of asparagus, and leaf spot of rubber	Phaeohyphomycosis (Agrawal and Singh, 1995)
Curvularia senegalensis	Seedling foliage blight on sugarcane (Rott and Comstock, 2000) and leaf spot of maize and other hosts (Yang, 1973)	Mycotic keratitis (Guarro et al., 1999)
Cylindrocarpon lichenicola	Postharvest fruit invasion and corm rot of *Colocasia esculenta* (taro) (Usharani and Ramarao, 1981)	Disseminated infection (Rodriguez-Villalobos et al., 2003) and keratomycosis (Mangiaterra et al., 2001)
Drechslera biseptata	*Drechslera* leaf spot of turfgrasses and black point in wheat grains (Fischl et al., 1993); mycotoxin producer (Leach and Tulloch, 1972)	Brain abscess (Mycology Online, 2004)
Fusarium chlamydosporum	Root rot and wilt of *Coleus forskohlii* (Boby and Bagyaraj, 2003) and blight of kangaroo paw (*Anigozanthos* spp.) (Satou et al., 2001)	Invasive infection (Segal et al., 1998)
Fusarium dimerum	One of several agents of fig endosepsis (Michailides et al., 1996)	Disseminated infection (Austen et al., 2001), endocarditis (Camin et al., 1999), and eye infection (Vismer et al., 2002)
Fusarium incarnatum	Walnut canker (Seta et al., 2004) and aster wilt	Mycotic keratitis (Naiker and Odhav, 2004); black gill disease of shrimp (Khoa et al., 2004)
Fusarium moniliforme	Ear, root, and stalk rot and seedling blight of maize (Westcott, 2001); sugarcane wilt complex; and pseudostem heart rot of banana (Jones and Lomeiro, 2000); wide host range	Human fusariosis, local and systemic (Dignani and Anaissie, 2004)
Fusarium oxysporum	Wilts and blights on a wide range of vegetable and plantation crops, ornamentals, small grains (Bottalico and Perrone, 2002), and turfgrasses, including potato, sugarcane, bean, cowpea, and *Musa* spp. (Raabe et al., 1981), and corm and root rots (Lucas and Dickinson, 1998)	Disseminated fusariosis (Sander et al., 1998), skin and nail infection (Romano et al., 1998), pneumonia (Rodriguez-Villalobos et al., 2002), and onychomycosis (Godoy et al., 2004)
Fusarium proliferatum	Leaf, sheath, stem spots, damping off, and flower spots on *Dendrobium* and *Cattleya* orchid; head blight in wheat and other small-grain cereals (Bottalico and Perrone, 2002); and wilt and dieback of date palm (Abdalla et al., 2000)	Disseminated infection in immunosuppressed individuals (Summerbell et al., 1988) and suppurative thrombophlebitis (Murray et al., 2003)

(TABLE 1 *continued*)

TABLE 1 *Continued*

Taxon[a]	Plant disease/association	Human disease
Fusarium solani	Yellows, fruit rots, seedling rots, root rots, and damping off on a wide range of hosts; fungal root rot of banana (Jones and Stover, 2000); and stem canker of sweet potato, black walnut, and poinsettia (Westcott, 2001)	Invasive furiosis (Repiso, et al., 1996; Bushelman et al., 1995) and onychomycosis (Godoy et al., 2004)
Lasiodiplodia theobromae	Fruit and stem rot of papaya (Dantas et al., 2003); canker of dogwood (Mullen et al., 1991); kumquat dieback (Ko et al., 2004); black kernel rot of maize; crown, finger, stalk, and peduncle rot of banana (Abeywickrama et al., 2004); and collar rot of peanut (Phipps and Porter, 1998)	Subcutaneous abscess (Maslen et al., 1996), ophthalmic mycoses (Thomas, 2003), onychomycosis, and phaeohyphomycosis
Lecythophora hoffmannii	Soft rots and decay of the surface layers of natural and preservative-treated timber (Bugos et al., 1988)	Chronic sinusitus (Marriott et al., 1997)
Paecilomyces variotii	Dieback and canker of pistachio (Ashkan et al., 1997)	Pneumonia (Byrd et al., 1992), central nervous system infection (Kantarcioglu et al., 2003), and peritonitis (Wright et al., 2003)
Phoma eupyrena	Blight of fir and pine species (Kliejunas et al., 1985)	Cutaneous lesions (Bakerspigel et al., 1981).
Phylum *Zygomycota*		
Mucor circinelloides	Fruit rot of *Luffa acutangula* (Singh et al., 1974) and mucor rot of mango (http://www.ismpminet.org/resources/common/names/mango.asp)	Zygomycosis (Chandra and Woodgyer, 2002) and gangrenous mucormycosis (Boyd et al., 2003)
Rhizopus oryzae	Fruit rots of pineapple, mango, and carrot (http://www.ismpminet.org/resources/common/names)	Pulmonary zygomycosis (Eisen and Robson, 2004)
Rhizopus stolonifer	Pre- and postharvest soft rots of many fruits, vegetables, and crops; sunflower head rot (Yang et al. 1979); and seedling blight on lupine (Westcott, 2001)	Zygomycosis (Gonzalez et al., 1996)

[a]Unidentified or inadequately identified species of *Microbacterium* and *Streptomyces* have been reported for clinical infections (Funke et al., 1997; Carey et al., 2001), and both identified and inadequately identified species have been associated with plants (Kaku, 2004; Zinniel et al., 2002; Westcott, 2001).

[b]Not included are zoonotic fungi, those from animals that can cause infections in people, fungal biocontrol agents, and commercial fungi used in brewing or baking. Members of these classes can cause human clinical disease. Toxigenic fungi and mycotoxins are not addressed.

[c]EPA, Environmental Protection Agency.

immunocompetent hosts. Sources of pathogens for microbial infection and contamination of plants include infected or infested seed, wind-driven inoculum, contaminated harvest machinery and containers, irrigation water, and postharvest handling (Scholthof, 2003). Humans can be exposed to microorganisms via any of these environmental sources. Exposure of humans is likely to increase during laboratory procedures of pathogen culturing and isolation. The airborne spores produced by fungi can initiate mycoses if inhaled by humans. By-products of plant pathogens in food, such as mycotoxins, can also cause illness (Bennett and Klich, 2003). Allergic reactions to plant pathogens and products such as toxins, while known to us, are not well documented (Hall et al., 1999). Allergens from some plant-pathogenic fungi, primarily *Alternaria* and *Fusarium* species, were included in a review by Horner et al. (1995).

With the prevalent paradigm of specificity among plant and human pathogens, it is not surprising that the literature is sparse on comparative connections among taxonomic groups of pathogens of different

TABLE 2 Selected fungal and viral gene homologues coding for virulence or pathogenicity factors in plant and human diseases

Gene(s)	Plant pathogen/host	Organisms known to cause disease or disease/malady
SP1; codes for extracellular protein (Hall et al., 1999)	*Stagonospora (Septoria) nodorum*; wheat blotch	*Coccidioides immitis, Aspergillus fumigatus*, respiratory allergenic proteins
Polymerases, RNase H, RNA-binding ZN finger, protease, primer-binding site (Hohn and Fütterer, 1997; Nault, 1997; Toh et al., 1983)	Cauliflower mosaic virus; many crop hosts; pararetroviruses	Retroviruses, hepadnaviruses, poliovirus
Helicase, protease, RNA-dependent RNA polymerase	Cherry rasp leaf virus (James and Upton, 2002; Thompson et al., 2004); Satsuma dwarf virus (Karasev et al., 2001)	Calici- and picornaviruses not known in humans, Equine rhinitis B virus, foot-and-mouth disease virus (Thompson et al., 2004)

hosts (Govan and Vandamme, 1998; Rahme et al., 1995; Hueck, 1998). Few studies have examined the capacity of microorganisms to cause disease in both plants and animals (Rahme et al., 1995; Wigley and Burton, 1999; Govan and Vandamme, 1998; Tan, 2002). It has long been recognized that certain plant-infecting viruses are able to propagate in insect vectors and that viruses in the same family (e.g., *Reoviridae* and *Rhabdoviridae*) are pathogens of vertebrates (Nault, 1997).

RISK POTENTIAL FOR LABORATORY PERSONNEL

As mentioned previously, healthy adults are not normally at risk of being infected by plant-associated microorganisms, but allergic reactions may occur. Good laboratory practices and some specific suggestions for dealing with plant-associated microorganisms are found in the methods manuals previously cited and the NIH guidelines. Immunocompromised adults, e.g., transplant recipients and those with immunodeficiencies (genetic or microbial), and persons with allergic sensitivities should take particular care in handling plant pathogens and microorganisms associated with plants. Large-scale cultures, aerosol-generating procedures, the use of needles and syringes, and direct contact with skin wounds are examples of activities that may increase the risk of exposure and infection.

CONTAINMENT

Samples of plant material obtained for isolation of pathogens or biocontrol agents are protected from contamination by using aseptic techniques, including surface sterilization of seeds, leaves, stems, and roots. In isolation procedures, materials may be ground or sliced to obtain the putative organism directly or after concentration in centrifuge tubes; buffers are usually added to such materials to obtain suitable suspensions and to provide optimal pH and ionic composition for stabilizing the structure of the pathogen. Some pathogens are obligate parasites or simply cannot be cultured; such organisms are not currently known to present an infectious risk to humans, but they may be allergenic. After microorganisms are isolated, experimental procedures frequently involve the generation of aerosols, e.g., by flaming, low- or high-pressure spraying, or inoculating plants with various mechanical devices. Plants may need to be wounded prior to or during inoculation with such materials as silicon carbide or Carborundum, which itself is a corneal and respiratory irritant.

Air-purifying particulate filter respirators that are effective against intake of particles 2 μm or larger from the surrounding air should be considered for all personnel and recommended for at-risk groups who may be exposed to potentially infectious or allergenic aerosols. The organisms known to have been associated with a disease condition in humans, and thus of particular concern, are listed in Table 1. The N95 NIOSH (National Institute for Occupational Safety and Health) series of filters are recommended. They are easy to wear, disposable, and of modest cost. In scientific supply catalogs, these filters have NIOSH approval numbers with the prefix TC-21C. Surgical masks are not suitable, since they fail performance criteria for protection against airborne contaminants. (Further information on respiratory protection may be found in chapter 17.)

The recommended containment level for all plant-associated microorganisms is BL1 or BL2 conditions in the laboratory or growth chamber, and BL1-P or BL2-P conditions in the greenhouse, as delineated in the NIH guidelines (NIH, 2002). These principles of containment are applicable to both wild-type and recombinant organisms and are design based to protect laboratory workers. When BL3-P conditions are required by regulators, they are for minimizing escape of the pathogen and for protection of the environment, not people.

Guidelines for using microorganisms in field work or natural ecosystems are provided by the USDA (1992) and can be found in the primary literature on plant pathology. The texts and methods manuals mentioned in the introduction may be consulted for organismal isolation, survival, growth, decontamination, and plant inoculation. Each organism-plant interaction is unique and requires special conditions of plant susceptibility, such as plant age, tissue specificity, temperature, humidity, and photoperiod, for achieving an infection which mimics natural conditions. Specific insect vectors may be necessary to inoculate plants with some viruses that cannot be mechanically transmitted. For several purposes, tobacco (*Nicotiana tabacum*) and the weed *Arabidopsis thaliana* are considered model plants for testing putative plant pathogens, akin to mouse models for human pathogens. There is only one documented case of endangerment of field populations of plants known to have occurred as a result of the use of plant pathogens in contained facilities (McKeen, 1989). Nevertheless, the USDA's Plant Protection Act regulations (section 7CFR330) require that unless a plant pathogen has been isolated locally, permits for its use must be obtained and containment conditions specified therein must be followed. These conditions are aimed at preventing damage to plants through environmental dissemination, rather than at protecting the human worker.

DISPOSAL

In the laboratory or greenhouse, autoclaving cultures and pathogen-infected material, or otherwise rendering them biologically inactive, is routine. In gardens and experimental fields, as well as commercial areas, timely chemical treatments for a few bacteria, many fungi, and some insect vectors or wild hosts decreases the inoculum. Chemicals are not available or cost-effective for controlling many plant pathogens, and biocontrol agents are few. Other management practices to decrease the inoculum, and thus decrease exposure, are crop rotation, planting of resistant varieties (where available), planting date, and plowing under infected or infested plant material. These practices decrease inoculum by the process of competition with other microorganisms in the soil, where many plant pathogens are poor survivors. Composting can also be effective.

MOVEMENT OF PLANT PATHOGENS

If the isolated microorganism is known to be a plant pathogen (e.g., see Agrios, 2005), irrespective of risk (no official risk groups are delineated for risk of a pathogen to plants or for risk to humans), a permit is required from the USDA-APHIS to move the agent from one location, state, or country to another. Packaging, storage, and transportation of plant pathogens are also under the aegis of APHIS rules and regulations (USDA, 2001), as well as the Department of Commerce. However, suspected or unknown pathogens can be sent by ordinary mail to laboratories for diagnostic purposes, as is done for human clinical specimens. (See also chapter 21 on shipping.)

CONCLUDING REMARKS

Greater caution should be exercised by those working with plant pathogens and biocontrol agents than has routinely been taken. Specifically, aerosol generation in plant and culture experimentation should be minimized or carefully controlled. In addition to concern about the etiologic agent, multiple antibiotic resistance is exhibited by some of these microorganisms, such as *B. cepacia* (Wigley and Burton, 1999) and *Stenotrophomonas maltophilia* (Denton and Kerr, 1998). For fungi, mortality from invasive infections is much greater than for bacterial pathogens (Engelhard, 1998), due to the limited treatment options available. Immunocompromised adults and persons with allergic sensitivities or open wounds should take particular care in handling plant pathogens and microorganisms associated with plants. Development of rapid and reliable methods for identifying viral, bacterial, and fungal pathogens will make diagnosis easier. New therapeutic approaches for treating invasive bacterial and fungal infections of humans, as well as virus-associated allergies, are future medical challenges.

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